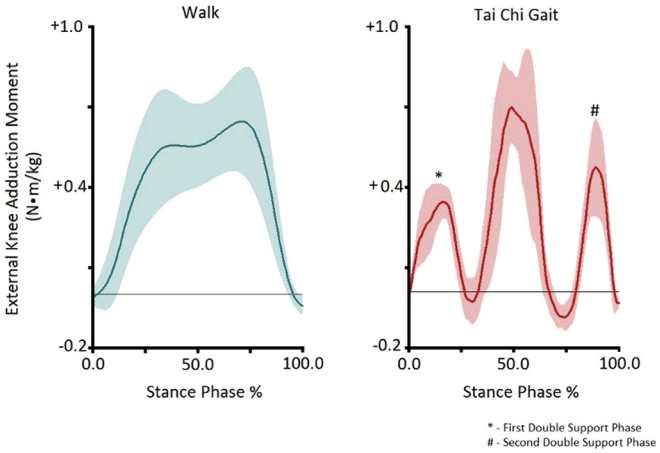
understanding how TCG incorporates different weight shifting strategies to create a beneficial loading response.



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GAIT METRIC PROFILE AND GENDER DIFFERENCES IN HIP OSTEOARTHRITIS PATIENTS. A CASE-CONTROLLED STUDY

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Purpose: Hip osteoarthritis (OA) is a slowly progressive destructive disease that results in alterations in joint loads and biomechanics to which patients adapt compensatory alterations and abnormal gait patterns. This prospective cross-sectional, case-controlled study examined these alterations in gait metrics and evaluated gender differences in gait spatiotemporal parameters. Correlations between function and gait metrics were also investigated.
Methods: Hip OA patients (38 females and 122 males) and healthy controls (14 females and 24 males) matched for age and gender underwent the same investigative protocol consisting of a spatiotemporal gait analysis followed by functional evaluations using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and the SF-36 Health Survey (SF-36).
Results: Differences between the patient and the control groups were significant in all the spatiotemporal parameters. There were significant gender differences within the hip OA group in all parameters except for cadence and single limb support percentage. WOMAC and SF-36 scores revealed significant differences between the study and control groups in most components. Significantly higher scores in the 3 components of the WOMAC as well as in 6 SF-36 score components were found among males compared to females in the patient group.
Conclusions: Gait, WOMAC and SF-36 were effective objective and subjective tools for evaluating a large cohort of patients with hip OA, and can be highly useful for supplementing the assessment of hip OA severity and enhancing treatment efficacy during the course of the disease.

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A NEW METHOD TO EVALUATE THE PROPRIOCEPTION IN KNEE OSTEOARTHRITIS: MULTISCALE ENTROPY

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Purpose: Introduce a new method to evaluate the proprioception in knee osteoarthritis, and compare it with the position sense tests and the motion sense tests which are the most commonly used measurement methods.
Methods: 24 patients with knee osteoarthritis whose Kellgren/Lawrence grade were grade3 or grade 4 and 12 healthy subjects underwent three tests of proprioception. Two tests were position sense test and motion sense test which were the most commonly used measurement methods. One test was a new evaluating method called multiscale entropy. The position sense test and the motion sense test measured proprioception using a test device which could accurate the absolute angular error to two decimal places. In the test of multiscale entropy, the subjects aimed the target sheet in sitting posture using their lower limbs for 60s, and the light spot locations were recorded by the camera. Then we utilized multiscale entropy to get the complexity of the light spot locations, which was used to evaluate proprioception. All subjects underwent these three tests at the legs' angle of 30°and 60°.
Results: There was a significant difference between the patients and the healthy subjects in the results of all three tests ($p < 0.01$). There was no correlation between the test of multiscale entropy and the motion sense test or between the test of multiscale entropy and the WOMAC score. There was, however, a significant correlation between the test of multiscale entropy and position sense test, especially at the angle of 60°($r = -0.629$, $p = 0.001$).
Conclusion: Proprioceptive accuracy of the knee is impaired in knee OA patients. The test of multiscale entropy can be used to evaluate the proprioception in knee osteoarthritis.

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SKELETAL EFFECTS OF MECHANICAL LOAD ARE SYSTEMIC AND MAINTAINED THROUGH FSTL3

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Purpose: Mechanical loading is required for bone remodeling and strengthening. We have previously shown that Follistatin-like 3 (FSTL3, NM_005860) mediates mechanical load-driven bone remodeling. Here we show that FSTL3 regulates bone remodeling by simultaneously regulating osteoblast and osteoclast activity *in vivo* and *in vitro*.
Methods: The Institutional Animal Care and Use Committee at The Ohio State University approved all protocols. Female Sprague Dawley rats (12-14 wks, n = 10), wild-type (WT) or homozygous *Fstl3*-/- C57Bl/6 mice (10-12 wks, n = 10/group) were exercised (EX) by treadmill walking at 12 m/min (rats) or 8 m/min (mice) for 45 min/day. Following 0, 2, 5 or 15 days of daily EX, animals were sacrificed, and gene expression was analyzed by quantitative real time polymerase chain reaction (qRT-PCR). The regulation of osteoblast and osteoclast activation by EX or FSTL3 was assessed by Western blot analysis or immunohistochemistry *in vivo* and *in vitro*. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc or t-test.
Results: Rapid *Fstl3* mRNA induction, reaching a 6 fold increase on day 2 and then declining to basal levels by day 5, occurred in osteoblasts and osteocytes derived from trabecular bone and bone marrow cells from healthy mice EX for 2 or 5 days. Similarly, a robust increase in FSTL3 protein expression, as assessed by immunofluorescence, was observed after 2 and 5 days of EX in rat femurs, predominantly within and adjacent to trabecular bones. However, upregulation of FSTL3 in osteoblasts was minimal in the control, non-EX trabecular bone or lining cells. Simultaneously, EX induced a rapid suppression in the number of multinucleated osteoclasts in the distal head of femurs in WT mice. Strikingly, such EX-induced suppression of osteoclast numbers was not observed in *Fstl3*-/- mice. Bone mineral apposition rate (MAR), as assessed by the deposition of calcein and alizarin complexone at the periosteal as well as endosteal surfaces of the femur, was significantly increased ($p < 0.003$) in response to EX in WT mice. However, an increase in MAR was not observed in *Fstl3*-/- mice exposed to EX. The representative stress-strain curves of the cortical bone of the WT and *Fstl3* -/- femurs under three point bending test also revealed that strain at ultimate strength of femurs from WT mice was approximately 25% greater than that of *Fstl3* -/- mice. Furthermore, the yield strain and yield energy were significantly upregulated by EX in WT, but not in *Fstl3*-/- mice. Finally, FSTL3 and dynamic compressive forces suppressed the differentiation as well as maturation of osteoclasts *in vitro*.
Conclusions: The actions of FSTL3 as a mechanoresponsive protein may involve both its upregulation of osteoblast functions as well as suppression of osteoclast functions. The discovery of FSTL3 as a

mechanosensitive protein may provide a new paradigm for investigating EX-driven bone formation and regulation of osteoblast and osteoclast activity. We propose that FSTL3 may also serve as a target to develop therapeutic drugs for treating bone diseases.

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BONE SECRETED DICKKOPF-RELATED PROTEIN 1 AMELIORATES OSTEOARTHRITIS IN MICE

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Objective: Cartilage loss and subchondral bone changes are hallmarks of osteoarthritis (OA). The family of the Wnt pathway is involved in the regulation of bone and cartilage homeostasis. Our preliminary results suggested that the Wnt/β-catenin pathway is mainly activated in bone during OA, but the effect of the reduction of bone Wnt pathway on cartilage remodeling are unknown. We here investigated the impact of the inhibition of the Wnt pathway specifically in bone tissue during the development of OA.
Methods: Joint instability induced by partial meniscectomy (MNX) was performed in mice to promote OA. We investigated the impact of the inhibition of bone Wnt pathway in OA development using mice overexpressing Dkk1 in bone (2.3 Col1-Dkk1Tg) after MNX. The effects of Dkk-1 in chondrocyte and osteoblast metabolism were further assessed using supernatant transfer and MMP expression.
Results: The Wnt/β-catenin pathway was activated in bone and cartilage, predominantly in osteocytes of subchondral bone and osteophytes, but remained weak in articular cartilage throughout the development of OA. The number of Dkk1 (+) chondrocytes was high at baseline (84.2 ± 3.1%) and decreased markedly during the course of OA from week 4 (14.4 ± 3.8%) to week 6 (5.7 ± 1.6%). At baseline, Dkk1-Tg mice had lower bone volume which was further reduced in MNX knees. Dkk1-Tg experienced a lower OA score than WT mice (5.1 ± 0.63 vs 8.4 ± 0.6, $p = 0.002$) independently of the expression of Dkk1 in chondrocytes. This was accompanied by a reduction of the subchondral bone and osteophyte volume. However, addition of supernatant of osteoblasts derived from Dkk-1-Tg mice or in vitro addition of rhDkk1 in chondrocytes promoted chondrocytic expression of MMP-3, -13 and ADAMTS-4 while the supernatant of pre-exposed osteoblasts with Dkk1 decreased the expression of proteases. Because Dkk1-Tg osteoblasts produced low VEGF, we tested whether VEGF could mediate the anticatabolic effect observed in vivo. Blocking VEGF in the supernatant of osteoblast cultures reversed the expression of MMPs by chondrocytes.
Conclusion: We confirm that Wnt is mainly activated in bone in OA joints. Inhibition of Wnt pathway by Dkk1 overexpression in bone decreased OA severity by reducing VEGF production. Targeting bone could be a useful approach for the treatment of OA.

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CHIP IS A CRITICAL REGULATOR OF TRAF6 DEGRADATION AND OSTEOCLAST FORMATION

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Purpose: Bone tissue is constantly remodeled. Abnormal osteoclast formation may result in bone disorders, such as osteoporosis, Paget's disease, and rheumatoid arthritis. Nuclear factor of κB (NF-κB) plays a key role in osteoclast formation and bone resorption. A key event in NF-κB signaling is the activation of an adaptor protein TRAF6. Once activated, TRAF6 further activates IKKβ (I-κB kinase), which phosphorylates I-κBα (the inhibitor of NF-κB), leading to its degradation. Carboxyl terminus of Hsp70-interacting protein (CHIP) is an E3 ligase and regulates the stability of several proteins which are involved in tumor growth and metastasis. However, the role of CHIP in bone remodeling *in vivo* has not been reported. The objective of this study is to investigate the role of CHIP in regulation of bone mass and bone remodeling and determine the molecular mechanism of CHIP in regulation of TRAF6 protein stability.
Methods: The bone phenotype of 1-month-old *Chip*$^{-/-}$ mice was examined by histology, histomorphometry, μCT, and gene expression analyses. The regulation of CHIP on TRAF6 degradation and the inhibition of NF-κB signaling was examined by immunoprecipitation, Western blotting, and ubiquitination assay.
Results: In this study, we found that deletion of *Chip* leads to osteopenic phenotype. There was significant reduction in bone volume (%, BV/TV, 66% decrease) and bone mineral density (59% decrease) in *Chip*$^{-/-}$ mice. Trabecular numbers (44% decrease), trabecular thickness (33% decrease) were significantly decreased in *Chip*$^{-/-}$ mice. In contrast, trabecular separation (1.7-fold increase) was significantly increased in *Chip*$^{-/-}$ mice. Higher structure model index (1.4-fold increase) and lower connectivity density (75% decrease) were observed in *Chip*$^{-/-}$ mice, suggesting more fragile bone when *Chip* gene is deleted. Cortical bone volume (40% decrease) and bone mineral density (40% decrease) were also significantly reduced in *Chip*$^{-/-}$ mice. One major reason for the osteopenic phenotype observed in *Chip*$^{-/-}$ mice could be due to the increase in osteoclast formation. TRAP-positive osteoclast numbers (1.6-fold increase) and osteoclast surface (1.5-fold increase) were significantly increased in *Chip*$^{-/-}$ mice. *In vitro* osteoclast formation assay showed that there was 3.5-fold increase in osteoclast formation in *Chip*$^{-/-}$ mice using osteoclast precursor cells isolated from WT and *Chip*$^{-/-}$ mice. Consistently bone resorption area was also increased in *Chip*$^{-/-}$ mice, demonstrated by an *in vitro* bone resorption assay using bone marrow cells derived from WT and *Chip*$^{-/-}$ mice. The expression of osteoclast marker genes, such as *Cathepsin K* (7-fold increase), *Mmp9* (16-fold increase), *Trap* (16-fold increase), and *Nfatc1* (3-fold increase), was significantly increased after RANKL treatment in bone marrow cells derived from *Chip*$^{-/-}$ mice. There was a significant increase in TRAF6 protein levels in *Chip*-deficient bone marrow cells. In contract, no changes in TRAF6 mRNA levels were found in the same *Chip*$^{-/-}$ cells, suggesting that CHIP may regulate TRAF6 protein stability. In a series of *in vitro* studies we found that: 1) Interaction of endogenous CHIP with TRAF6 was detected in RAW264.7 osteoclast-like cells. 2) The ubiquitination of endogenous TRAF6 protein was reduced in *Chip*$^{-/-}$ bone marrow cells. 3) The phosphorylation of IKKα/β and IκBα was significantly increased in *Chip*$^{-/-}$ bone marrow cells after RANKL stimulation. 4) p65 nuclear translocation was enhanced in *Chip*$^{-/-}$ bone marrow cells when the cells were treated with RANKL.
Conclusions: Our studies demonstrated that CHIP interacts with TRAF6 in osteoclast precursor cells and promotes TRAF6 ubiquitination and proteasome degradation; subsequently inhibits NF-κB signaling and regulates osteoclast formation. In *Chip* KO mice TRAF6 protein levels are increased, leading to activation of osteoclast formation and significant bone loss.

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SPATIAL ASSOCIATION OF SUBCHONDRAL OSTEOSCLEROSIS WITH ENHANCED MARROW IMMUNE CELL INFILTRATION, OSTEOCLAST ACTIVITY AND CARTILAGE DEGENERATION IN HUMAN OSTEOARTHRITIS

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Purpose: Osteosclerosis of subchondral bone is a pathological hallmark of osteoarthritis (OA) and increasing evidence supports a pivotal and active role of this tissue in the initiation and progression of disease in experimental and human OA. The cellular and molecular regulation of subchondral osteosclerosis remains poorly understood. Regulation of bone remodeling by immune cells, termed osteoimmunology, has been demonstrated in a number joint disorders including rheumatoid arthritis and osteoporosis. In this study we investigated whether osteoimmunological mechanisms might be involved in regulating subchondral osteosclerosis in human OA.
Methods: Subchondral bone mineralization density (BMD) of explanted OA tibial plateaus was mapped using computed tomography osteoabsorptiometry (CT-OAM) analysis. Areas having a subchondral BMD below 800 or over 1200 Hounsfield units were defined to be non-sclerotic and sclerotic, respectively. Using CT-OAM mapping, histological tissue sections were prepared from both areas ($n = 18$ each). Cartilage degeneration and subchondral bone area fraction were determined using Mankin score and the ImageJ-plugin BoneJ, respectively. Presence of immune cells in subchondral bone marrow tissue was investigated using immunohistological and flow cytometry analyses for expression of CD3 (T-lymphocytes), CD20 (B-lymphocytes) and CD68 (macrophages). Functional osteoclasts were identified by histochemical staining for tartrate-resistant acid phosphatase (TRAP) activity. Outgrowth cultures of nonsclerotic and sclerotic bone were stained for TRAP and alkaline phosphatase (ALP) activity. The effect of conditioned medium from nonsclerotic and sclerotic subchondral bone